Controlling specific and non-specific binding of proteins at diamond surfaces via covalent functionalization with molecular monolayers

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Keywords: proteins, non-specific binding, diamond, covalent functionalization.

Abstract

INTRODUCTION

The non-specific binding of proteins at surfaces degrades the performance of surface-based biosensors and is also relevant for applications such as biomedical implants. Molecules containing repeated ethylene glycol

(-O-CH_2-CH_2-) units have been shown to resist the non-specific bonding of many proteins. While long EG polymers can be used in many applications, for most surface-based biosensing applications one desires to have a molecular layer that inherently resists non-specific binding, and then to embed within that layer specific molecules that have a high affinity for binding specific proteins of interest. In such applications, one desires to control the ratio of specific binding (to the target of interest) to non-specific binding (to other molecules) in a complex mixture of proteins. One way of accomplishing this is to use mixed monolayer consisting of shorter EG oligomers, mixed with specific recognition elements of interest to form a monolayer film.

Previous studies have shown that short oligomers of ethylene glycol consisting of only thee ethylene glycol units ("EG3") can be effective at reducing non-specific binding of proteins to gold surfaces. There has recently been great interest in the use of diamond as a platform for biosensing,[1-3] mandating a desire to understand how to control the specific and non-specific binding of proteins by controlling the structure and chemical composition of the diamond surface.. In this study, we have investigated how covalent functionalization of diamond surfaces with short ethylene glycol oligomers containing between 3 and 6 EG units affects the non-specific binding of four model proteins to the surface.[4-5] Studies have been performed using avidin, fibrinogen, bovine serum albumin, and casein as model proteins. We also used three different types of diamond samples: polycrystalline diamond thin films grown on silicon substrates, high-pressure high-temperature diamonds, and a natural single-crystal diamond, to help identify how surface roughness affects the non-specific binding. Finally, by mixing these EG oligomers with specific biomolecular recognition elements (such as avidin, which has a strong affinity for avidin), one can maximize the surface chemical composition to maximize the specific binding of proteins to the recognition elements of interest, while simultaneously minimize the non-specific binding of other proteins. Ultimately, our work demonstrates how to optimize the diamond surface structure and chemistry to control the specific and non-specific binding of proteins to the surface.

COVALENT MODIFICATION WITH ETHYLENE GLYCOL OLIGOMERS

We previously developed a method for covalent functionalization of diamond surfaces using molecules bearing a vinyl (C=C) group at one end of the molecule. To covalently link biomolecules such as DNA to the diamond surface, it is possible to use molecules in which one end is terminated with a vinyl group and the other end of the molecule is terminated with an amine or other suitable chemical functional group. Here, we use molecules with a vinyl group at one end and ethylene glycol oligomers at the other end.

The non-specific binding was measured in two methods. In the first method, the surfaces were exposed to fluorescently-tagged proteins of interest. After copious rinsing, the amount of residual protein on the surface was measured using a fluorescence imager. Because fluorescence quenching at surfaces can significantly affect the fluorescence intensity, this method cannot be used for quantitative measurements or to compare different substrates, such as diamond and gold, that would be expected to exhibit very different amounts of quenching. Consequently,

quantitative measurements were performed using a wash-off method in which the protein bound to a surface is removed via chemical digestion and extensive soaking in surfactant, and the fluorescence intensity of this wash-off solution is measured with a sensitive fluorometer and compared with standards of known concentration.

Since amine groups are often used for covalent attachment of biomolecules to surfaces, we compared the EG-modified surfaces with those functionalized with primary amine groups. Our experiments show that chemical modification with the short EG3 oligomer reduces non-specific binding on polycrystalline diamond samples by approximately 70% compared with the amine-terminated surface. Our data show that functionalization of diamond with EG3 is very effective at reducing non-specific binding for all four proteins investigated. However, we find additional reduction by extending the EG moiety from 3 to 6 unit, as the EG6 molecule reduces the non-specific binding of aviding by 98%, and by more than 90% for all four proteins investigated. For comparison, we have also performed experiments using dodecene, which produces a surface functionalized with simple alkyl chains. While dodecene also helps to reduce non-specific binding for some proteins, it is significantly less effective than EG3 or EG6.

By mixing EG6 with a vinyl-terminated protected amine, it is possible to fabricate mixed monolayers of controlled composition on the diamond surface. By measuring the amount of specific and non-specifically bound protein as a function of monolayer composition, we investigated how to optimize the monolayer composition to maximize the ratio of specific/non-specific binding. For the case of avidin-biotin, we find that using a mixture containing approximately 70% EG6 and 30% amine groups produces more than a factor of 12 improvement in the overall ratio of specific to non-specific binding.

Finally, we compared the reduction of non-specific binding on diamond surfaces with different microscopic morphologies. Quantitative measurements show that EG6-functionalized polycrystalline diamond samples resist non-specifically bound proteins to about the same extent as functionalized surfaces of gold self-assembled monolayers. Since gold-SAM structures are widely used, this indicates that functionalized diamond surfaces are at least as good as gold-SAM structures. However, in experiments using a (111)-cleaved natural single-crystal diamond, we find an additional significant reduction. The EG6-modified single-crystal diamond sample is significantly more effective at resisting non-specific binding than any other sample we have measurent. These results indicate that the microscopic roughness present on microcrystalline diamond samples contributes significantly to the residual non-specific binding. Thus, efforts to reduce the nanometer-scale roughness of diamond surfaces would be expected to yield further reduction in non-specific binding. For biosensing applications, this in turn leads to improvements in sensitivity and selectivity.

Our results show that the behavior of diamond films can be significantly modified using molecular monolayers consisting of short oligomers of ethylene glycol. Photochemical modification of the surface using using ethylene glycol oligomers with a terminal vinyl group provides a mean to achieve this. Diamond surfaces covalently modified in this way are highly effective at resisting protein binding, while the use of mixed monolayers provides a way to add specific biomolecular recognition elements at will. Our results demonstrate a clear pathway for producing well-defined molecular layers exhibiting precisely-tailored biological interactions.

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